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Bioaccumulation of Kepone by Spot ('Leiostomus xanthurus'): Importance of Dietary Accumulation and Ingestion Rate

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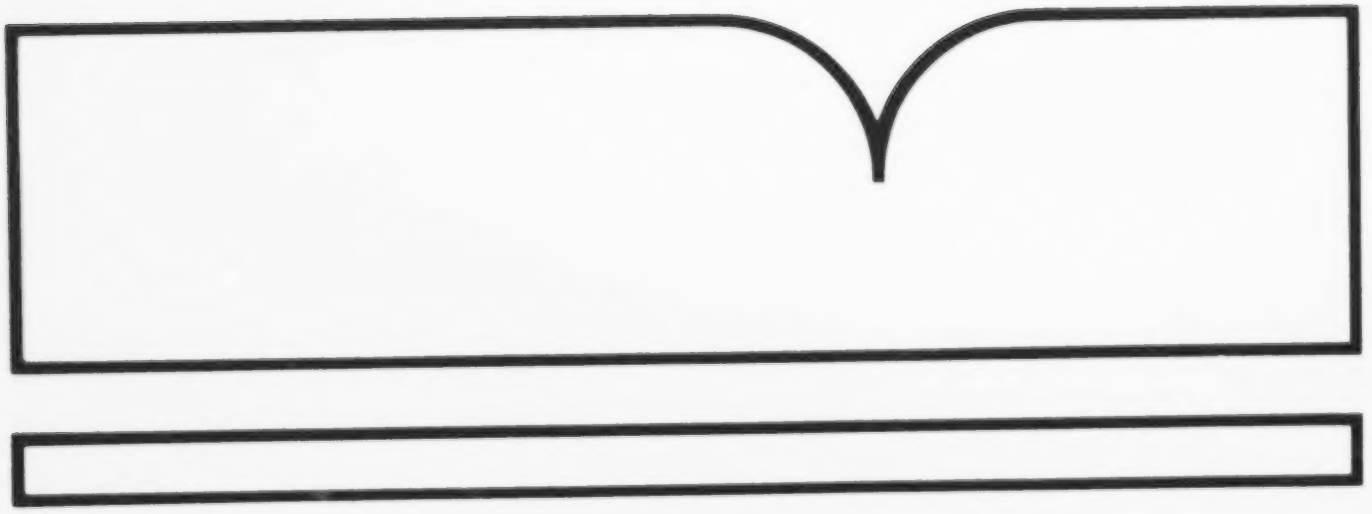

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16. ABSTRACT

The relative extent of dietary accumulation and bioconcentration of Kepone by spot (Leiostomus xanthurus) was quantitatively evaluated at food rations of 4, 8 or 20% of the average wet weight of fish. ^{14}C-Kepone was utilized to determine bioconcentration and dietary accumulation separately, while ^{14}C-Kepone-contaminated food (grass shrimp, Palaemonetes pugio) and unlabaeled Kepone in water were used to determine simultaneously accumulation from both sources. Grass shrimp and spot were exposed to the same aqueous Kepone concentration (0.04 µg/ℓ). A first-order pharmacokinetic equation was used to model Kepone accumulation kinetics during the 19-day uptake and 28-day clearance phases.

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BIOACCUMULATION OF KEPONE BY SPOT (*LEIOSTOMUS XANTHURUS*): IMPORTANCE OF DIETARY ACCUMULATION AND INGESTION RATE

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The relative extent of dietary accumulation and bioconcentration of Kepone by spot (*Leiostomus xanthurus*) was quantitatively evaluated at food rations of 4, 8 or 20% of the average wet weight of fish. [^{14}C]Kepone was utilized to determine bioconcentration and dietary accumulation separately, while [^{14}C]Kepone-contaminated food (grass shrimp, *Palaemonetes pugio*) and unlabeled Kepone in water were used to determine simultaneously accumulation from both sources. Grass shrimp and spot were exposed to the same aqueous Kepone concentration (9.04 μg/l). A first-order pharmacokinetic equation was used to model Kepone accumulation kinetics during the 19-day uptake and 28-day clearance phases. A doubling of contaminated food ration caused a doubling of the whole-body Kepone concentration in spot. Spot fed an 8% ration of uncontaminated food and exposed to aqueous Kepone did not bioconcentrate significantly greater amounts of the pesticide than fish fed a 4% ration and exposed to the same aqueous concentration. When spot were exposed to contaminated water and food, Kepone contributions from each source were additive. Feeding rate, however, was very important in determining final Kepone body burdens in spot. The dietary source of Kepone represented approximately 9, 18 and 37% of the total body burden bioaccumulated by fish fed 4, 8 and 20% food rations, respectively, but assimilation efficiencies of Kepone from the food source were low. The laboratory results further suggest that dietary accumulation of Kepone by spot may play an important role in determining final Kepone concentrations in spot in the James River, Virginia.

Key words: Kepone; Dietary accumulation; Bioconcentration; Assimilation efficiency; Ingestion rate; *Leiostomus xanthurus*

INTRODUCTION

The release of the organochlorine pesticide Kepone (chlordecone) into the James River, Virginia, has caused extensive contamination of the water, sediment and biota of this estuarine system (Huggett and Bender, 1980). The extent and persistence of this pollution has led to the generation of a large data base describing Kepone toxicity, bioaccumulation and distribution. These data provide, in part,

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basis for an evaluation of how well hazard assessment techniques work for an estuarine system.

Bender et al. (1984) attempted to correlate measured aqueous Kepone concentrations from the James River to residues in aquatic organisms, utilizing laboratory-derived bioconcentration and dietary accumulation factors. They found that, in many cases, correlations were good. For example, when laboratory-derived dietary accumulation factors (Fisher et al., 1983) for male blue crabs (*Callinectes sapidus*) were applied to field exposure concentrations, adequate predictions of measured Kepone residue concentrations in feral crabs were obtained. However, major discrepancies between laboratory bioconcentration estimates and field-residue measurements were shown for bottom-feeding fishes, including spot and Atlantic croaker (*Micropogonias undulatus*). Several explanations are possible for these discrepancies: (1) experimentally derived bioconcentration factors were too low; (2) aqueous concentrations of Kepone in the James River were greater than measured; (3) direct input from sediment increased the total body burden; or (4) significant input of Kepone from the diet of fish increased the body burden.

Bender et al. (1984) reviewed the aqueous Kepone concentration data from the James River and found them to be in agreement with the 5 to 7 ng/l concentrations predicted by sediment-water partition coefficients (Slone and Bender, 1980; Strobel et al., 1981). The bioconcentration factors used by these investigators were the maximum found for any fish species (Hansen et al., 1977), rather than the lower value for spot determined by Bahner et al. (1977). Thus, it appears that the authors may have overestimated Kepone bioconcentration. Moreover, Bahner et al. (unpublished) reported that uptake of Kepone from water was more important for spot than uptake through contact with, or ingestion of, sediment. This leaves significant dietary uptake of Kepone as the most obvious explanation for the differences between predicted and measured levels of Kepone in spot.

One objective of the present research was to determine the possible significance of dietary accumulation of Kepone to final residual body burdens in spot. Macek et al. (1979) have reviewed available literature on Kepone dietary accumulation and concluded that its input to fish would be insignificant. We believed that there were enough questions concerning the Kepone literature and its applications by Macek et al. (1979) to warrant a definitive study. We also wished to investigate the effect of food consumption on the uptake of Kepone by spot. We believed that early studies concerning the importance of dietary accumulation of lipophilic compounds may have been unconsciously biased due to small rations fed to test organisms.

MATERIALS AND METHODS

General protocol

The test organisms were juvenile spot and non-ovigorous grass shrimp

(Palaemonetes pugio) collected from the estuarine waters of Santa Rosa Island adjacent to the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida (lat. 30°19′30″N, long. 87°10′45″W). Organisms were acclimated to experimental conditions in the test apparatus for 10 to 14 days prior to the start of the experiments. All tests were conducted between May and July, 1984. Spot used in the 4 and 8% ration study averaged 2.49 g ($\pm$0.30 SE, n = 416) wet weight at the start of the test, while those in the 20% ration study averaged 3.13 g ($\pm$0.46 SE, n = 120). Spot used for the assimilation efficiency studies weighed an average of 2.35 g ($\pm$0.64 SE, n = 60).

The [^{14}C]Kepone (24.8 μCi/μM) used in these experiments was obtained from Pathfinder Laboratory (St. Louis, MO)[1]. The unlabeled Kepone was obtained from the U.S. EPA Pesticides and Industrial Chemicals Repository (Research Triangle Park, NC). Both labeled and unlabeled Kepone were assayed 98% pure by gas chromatography. All aqueous exposures were conducted with a triethylene glycol carrier (0.03 ml/l).

Grass shrimp were exposed to 0.04 μg/l [^{14}C]Kepone in filtered sea water in a 15 l/h flow-through system (60 l tanks) for 16 days. A 10 ml/day Kepone stock solution was delivered to each of six tanks by syringe pump. Water temperature was maintained at 23°C and a 14L : 10D lighting cycle was maintained. Salinity was not controlled, but was monitored throughout the exposure. These same exposure and dosing conditions were used in the later spot experiments. Individual grass shrimp samples analyzed at the end of the exposure showed no significant difference in Kepone concentrations among tanks (ANOVA, α = 0.05). All [^{14}C]Kepone exposed shrimp were pooled, ground to a fine consistency and frozen for use in the feeding experiments with spot. The food was finely ground to allow for a more even distribution of Kepone among the test spot. This food averaged 0.101 μg [^{14}C]Kepone/g tissue ($\pm$0.003 SE, n = 10).

The experimental protocol used to determine relative contributions of Kepone to spot by bioconcentration and dietary accumulation was that outlined in Macek et al. (1979). Frozen food was weighed each day, chopped and transferred to jars containing seawater, where it was allowed to thaw before addition to the treatment tanks. Actual food ration was adjusted throughout the experiment for fish weight-gain and loss due to sampling. Grass shrimp which contained no detectable levels of Kepone were ground and used to feed control fish. The flow-through apparatus and test procedures were consistent with ASTM guidelines for conducting bioconcentration tests (ASTM, 1985). Kepone uptake and clearance were determined at daily food rations of 4 and 8% mean spot weight, while uptake alone was measured using a 20% ration.

At the 4 and 8% rations, 52 spot were tested in each of four treatments. The four

[1] Mention of commercial products does not imply endorsement by the U.S. Environmental Protection Agency.

treatments were: (1) 0.04 μg/l [^{14}C]Kepone-contaminated water + uncontaminated food to measure bioconcentration from water (4W & 8W [4 and 8% ration with water (W) exposure]); (2) uncontaminated water + [^{14}C]Kepone-contaminated food to measure dietary accumulation from food (4F & 8F); (3) 0.04 μg/l unlabeled Kepone-contaminated water + [^{14}C]Kepone-contaminated food to measure contributions from each source in a combined exposure (4FW & 8FW); (4) uncontaminated food as a control (4C & 8C). At the 20% ration only a [^{14}C]Kepone-contaminated food exposure was used. Results from Treatment (3) made it possible to differentiate between the two sources of Kepone and determine whether their contributions were additive. Secondary uptake of [^{14}C]Kepone excreted by the spot or leached from food could play a role in Kepone accumulation by fish. To monitor this possible route of uptake, a fine-mesh Nitex basket containing four fish was installed in the 20% contaminated ration treatment. These fish, which received clean food during the uptake phase, were analyzed for [^{14}C]Kepone and compared to fish which had fed on contaminated food in the same tank.

The exposure phase of the experiment lasted 19 days, followed by a 28-day clearance phase. In accordance with ASTM guidelines (ASTM, 1985), samples were taken during the uptake phase at geometric intervals, starting at day 0.88. Clearance samples were taken at arithmetic intervals, beginning with day 19. Fish were sampled prior to feeding. This allowed a 24 hour period for purging of the GI tract. Four fish were collected from each treatment per sampling period, when possible. They were removed, rinsed, blotted dry and killed by pithing. Whole body wet weights and standard lengths were recorded and the fish were frozen for subsequent analysis. These data allowed for the calculation of a fish condition index *(K)* by the formula (Parker, 1971):

$$K = \frac{(\text{Weight})\ (10^5)}{\text{Length}^3}.$$

All Kepone concentrations in tissues are expressed as μg Kepone/g whole-body, wet weight. Fish sampled at the start and end of the uptake and clearance phases were analyzed for total lipids, neutral lipids, glycolipids and phospholipids.

Assimilation efficiency experiments were conducted at 23°C and ambient salinity, using [^{14}C]Kepone-contaminated food. One-gallon pickle jars with two screened holes near the bottom were used as test chambers. The screen allowed for water exchange, but retained organisms and their food. The jars were placed in a 60 l tank receiving 15 l/h filtered sea water. A variable level overflow siphon in the large tank regulated timed flushing of the pickle jars.

Several different treatments were utilized in the assimilation experiments. One group of fish ($n = 4$) were fed a 4 or 8% ration of contaminated food and were analyzed after 24 h. Another group of fish received the same rations, but were analyzed after 3 h to determine Kepone ingestion efficiency. A third group of fish were isolated from their fecal material after being fed an 8% ration. Static (3 h) ex-

periments were also utilized to determine the partitioning of Kepone from the food source to the water. A more coarsely ground food was also used in a 24 h assimilation test and a static test (3 h) to determine if the grinding of the original food may have resulted in a significant loss of Kepone to the water.

Analysis of water and tissue samples

Both radiolabeled and unlabeled aqueous Kepone samples (1 liter) were extracted by adding 25 ml of toluene, shaking for 3 min and sampling from the toluene layer after 1 h. For the labeled Kepone, a 5 ml portion of the toluene was pipetted into 15 ml of PCS scintillation cocktail and counted on a Beckman LS 8000 liquid scintillation spectrophotometer for 10 min or until a 2σ error of 2% was attained. Replicate spiked water samples indicated a recovery efficiency with this method of 94.8% ($\pm$0.4 SE). A portion of the toluene extract could be injected directly into the gas chromatograph (GC) without clean-up (GC conditions same as for tissue samples). Radiometric and gas chromatographic analysis of duplicate [^{14}C]Kepone samples showed that the two methods gave equivalent estimates of Kepone concentration. The radiometric method gave a concentration of 0.042 μg/l ($\pm$0.002 SE, $n = 10$), while the GC method gave 0.041 μg/l ($\pm$0.003 SE, $n = 10$).

Whole fish and shrimp samples were analyzed for radiolabeled Kepone with an automated sample oxidizer. Fish were freeze dried before combustion. Samples were counted as above. Recovery efficiencies were 97.6% ($\pm$0.3 SE, $n = 10$) for the wet shrimp samples and 92.0% ($\pm$2.8 SE, $n = 12$) for the freeze-dried fish. All Kepone concentrations were corrected for background, recovery efficiency and quench.

Samples of fish from the 4FW and 8FW treatments were analyzed by a combined gas chromatographic/radiometric methodology. Frozen samples were thawed, homogenized and extracted three times with 1:3 toluene/ethyl acetate after soaking 24 h in the same extraction solvent. The samples were centrifuged each time and the extract decanted. After the combined extract was dried under nitrogen, 30 ml petroleum ether plus 5 ml fuming sulfuric acid were added. This mixture was shaken for 15 min, centrifuged for 15 min and the petroleum ether was decanted. The procedure was repeated twice with 10 ml washings of petroleum ether. The combined petroleum ether extract was dried under nitrogen and redissolved in 1 ml of 1:19 acetone/benzene. The sample was applied to a 300 mm by 11 mm (i.d.) oven dried Florisil column that had been rinsed with benzene. The column was then washed with 50 ml of 1:19 acetone/benzene, followed by 70 ml of 1:9 methanol/toluene. The last eluate was collected, evaporated, and redissolved in an appropriate volume of 2% methanol in toluene depending on the estimated Kepone concentration. One half of the final volume was counted to determine radioactive Kepone, while the other portion was analyzed on a HP5840 GC with EC detector (^{63}Ni), using a 5% SE-30 on Chomosorb w/AW DCMS column (1.83 m$\times$2 mm i.d.). Injection temperature was 275°C, with a P-10 flow rate of 30 ml/min. Recovery efficiencies

for spiked samples averaged 51.5% (± 13.8 SE, $n = 12$). All tissue data were corrected for recovery efficiency prior to comparison with radiochemical data. The low and variable recovery efficiency was apparently due to problems with lipid interference. Because recovery efficiencies for the [^{14}C]Kepone analysis were much higher and less variable, results from the experiments using only radiolabeled Kepone are given greater emphasis in the following discussion. Although the GC method was more variable, it is interesting that bioconcentration curves generated by BIOFAC are similar, regardless of the analytical method (Fig. 2).

Lipid samples were analyzed by a modified Bligh-Dyer method from Kates (1972). Lipids were extracted with a water/chloroform/methanol solvent mixture (0.8:1:2). After extraction, the lipids were dried and weighed in tared tubes. The total lipids were eluted through a column containing Unisil (Clarkson Chemical Co., Williamsport, PA). Neutral lipids were eluted with chloroform, glycolipids with acetone and phospholipids with methanol.

Statistical analysis

Spot growth, condition index, assimilation efficiencies and percentage lipid data were analyzed by a one-way ANOVA followed by a Duncan's Multiple Range test. An arcsine transformation was applied to all percentage data before analysis. Effects of ration size on Kepone concentration at each sampling period were evaluated by a *t*-test. The probability level of significance, unless otherwise stated, was 0.05. All statistical analyses were performed by using the SAS statistics program (1982).

Pharmacokinetic equations

The uptake and clearance data from this study were modeled by using the BIOFAC computer program developed by Dow Chemical Company (1978). This program uses a nonlinear maximum likelihood method to generate uptake and clearance curves from data that fit a first-order pharmacokinetic model. From these curves, the program calculates rate constants for uptake and clearance in addition to measures of half life and time to steady state. This type of model has been shown to describe the accumulation of many nonmetabolizable pollutants by aquatic organisms (Blau et al., 1975; Blanchard et al., 1977; Branson et al., 1975; Bruggeman et al., 1981; Hamelink, 1977; Pizza and O'Conner, 1983). The equation that describes bioaccumulation is of the form:

$$dC_o/dt = K_3Q + K_1C_W - K_2C_o \quad (1)$$

where: C_o = concentration in organism; Q = daily dietary dose = C_f (wt food offered × fish wt^{-1} × time^{-1}); C_f = concentration in food; C_W = concentration in

water; K_1 = uptake rate constant from water (day^{-1}); K_3 = uptake rate constant from food (day^{-1}); K_2 = clearance rate constant (day^{-1}).

For a constant aqueous concentration without dietary exposure, integration of Equation (1) yields the equation for determining the concentration in the organism at time *(t)* after the start of exposure:

$$C_o(t) = K_1/K_2 \cdot C_w \cdot (1 - e^{-K_2 t}). \quad (2)$$

Likewise, for a constant food concentration without water exposure, the concentration in the organism at time *(t)* can be given by:

$$C_o(t) = K_3/K_2 \cdot Q \cdot (1 - e^{-K_2 t}). \quad (3)$$

The daily dietary dose represents the total amount of contaminant offered the organism while the uptake rate constant (K_3) is a measure of the xenobiotic assimilation efficiency from food (Bruggeman et al., 1981).

In both Equations (2) and (3), the same clearance rate constant is used, under the assumption that once the compound is in the tissues, it will be eliminated at the same rate, regardless of the original source. A further assumption that allows for the above integrations is that the exposure concentration and rate constants are actually constant. One of the major advantages of this first-order model is that a steady state accumulation factor can be calculated without reaching a steady state plateau. The Dietary Accumulation Factor (DAF) and the Bioconcentration Factor (BCF) are simply the ratio of the uptake and clearance rate constants for that specific route of exposure. The DAF computed from these equations is more ecologically realistic than the Bioaccumulation Factor used by Bahner et al. (1977) since the former includes a feeding rate factor missing in the latter.

RESULTS AND DISCUSSION

Aqueous Kepone concentrations remained relatively constant during the food ration experiments (0.043 $\mu g/l \pm 0.003$ SE, $n = 39$). The mean water temperature for the 4% and 8% ration size study was 23.1°C (± 0.4 SE) while temperature in the 20% ration treatment averaged 22.9°C (± 0.2 SE). There were no significant differences in the water quality parameters monitored among treatments. Average values for salinity, dissolved oxygen and pH were 26.2‰ (± 1.9 SE), 6.3 mg/l (± 0.7 SE), and 7.65 (± 0.11 SE), respectively.

A 4% ration is considered a maintenance ration for many fishes (Hastings, 1969). Spot fed a 4% ration in the present study either just retained their initial weight or showed a weight loss over the experimental period (Table I). In contrast, spot fed an 8% ration gained weight, although in only one instance was the weight gain significant (Treatment 8W). Fish in this treatment were also significantly larger than

TABLE 1

Mean weight (WT), standard length (SL), percentage moisture (%M), condition index (CI) and weight change over time (WC,g) for spot during ration experiment (± SD).

Treatment[a]	day 0	day 19 – end uptake					day 47 – end clearance					
	WT,g	WT,g	SL,mm	%M	CI	WC	WT,g	SL,mm	%M	CI	WC	
							0–19				19–47	0–47
Control (4C)	2.53(0.29)	2.39(0.18)	52(1)	–	1.66(0.07)	−0.14	2.95(0.26)	53(1)	–	1.95(0.07)	0.56	0.42
Control (8C)	2.45(0.31)	2.54(0.53)	52(3)	–	1.77(0.12)	0.09	3.03(0.48)	54(3)	–	1.87(0.13)	0.49	0.58
Food only (4F)	2.49(0.29)	2.16(0.24)	51(3)	82.0(3.4)	1.63(0.12)	−0.33	2.60(0.36)	53(3)	80.2(0.5)	1.80(0.22)	0.44	0.11
Food only (8F)	2.41(0.32)	2.15(0.26)	50(2)	80.7(1.2)	1.69(0.07)	−0.26	2.46(0.53)	51(3)	78.3(0.8)	1.83(0.18)	0.31	0.05
Water only (4W)	2.50(0.30)	2.08(0.45)	50(3)	82.6(2.4)	1.62(0.09)	−0.42	2.49(0.46)	51(2)	80.0(1.0)	1.86(0.22)	0.41	−0.01
Water only (8W)	2.55(0.31)	2.51(0.43)	52(2)	81.2(1.8)	1.75(0.11)	−0.04	3.19(0.45)	55(3)	78.7(0.9)	1.93(0.11)	0.68	0.64
Food & Water (4FW)	2.49(0.32)	2.05(0.17)	49(1)	–	1.73(0.17)	−0.44	2.44(0.59)	51(3)	–	1.78(0.21)	0.39	−0.05
Food & Water (8FW)	2.47(0.28)	2.18(0.29)	51(2)	–	1.68(0.09)	−0.29	2.74(0.31)	53(3)	–	1.87(0.14)	0.56	0.27

[a]Exposure regime. Number represents ration.

spot in Treatment 4W. Spot fed an 8% ration also had a lower percentage moisture and, in most cases, a better condition index than spot fed the smaller ration, although only the differences in percentage moisture were significant. It should be noted that the condition index values during these experiments were not significantly different from those for a wild population of spot captured prior to the tests, which had a mean condition index of 1.93 (± 0.12 SE, $n = 54$).

Statistical analysis of wet weights and standard lengths of spot indicated that only at the end of the clearance phase were there significant differences among treatments. Most of the difference seen at this time could be traced to the fish in Treatment 8W (Kepone in water – 8% ration) which were larger and longer than fish in the other treatments (Table I). During the experiment, spot exposed to Kepone in any form tended to grow less than control fish, although these differences were not significant. This could have been due to a number of reasons, including an avoidance of the contaminated food, a difference in food particle size between contaminated and uncontaminated food, or some interference by Kepone with normal digestive processes. It must be remembered that this experiment was not designed as a growth study. The weight change value reported in Table I is a single value with no indication of variation (mean weight of sample at day 19 minus the mean weight at time 0). Any attempt to correlate reduced growth and Kepone exposure would be mere conjecture at this time, especially considering the rapid growth of fish exposed to Kepone in water and fed an 8% ration (Table I).

Spot at the start of the 4% and 8% ration experiment had a mean percentage total lipids of 4.2 (± 1.1 SE), based on dry weight (Table II). At the end of the uptake phase, only spot from Treatment 8W had significantly greater percentage total lipids than at the start. These same fish had significantly greater percentage total lipids than fish from all other treatments at this time. At the end of the clearance phase, all fish had significantly greater percentage total lipids than at the start, except those in Treatment 4W (Table II). In most cases, fish fed an 8% ration had greater percentage total lipids than those fed a 4% ration, but the differences among treatments were not significant. There were never any significant differences between percentage constituent lipids among treatments at any sampling period (Table II).

Neutral lipids represented on average 4.4% of the total lipids in these spot. This low percentage neutral lipids suggests that the 4 and 8% ration were close to a maintenance diet and very little neutral lipids were stored in adipose tissue. In contrast, spot fed a 20% ration of the same food preparation had a greater percentage total and neutral lipid content, indicating some storage of neutral lipids (Table II). These lipid data indicate that as fish were fed a larger ration they became healthier, and that a 4% ration of this food preparation was probably below a maintenance level.

A doubling of the contaminated food ration from 4 to 8% led to a doubling of the spot whole-body Kepone concentration, a significant increase (Fig. 1). Fish fed the 8% ration accumulated 0.027 μg Kepone/g tissue (± 0.004 SE, $n = 9$) by the end

TABLE II

Mean percentage total lipid, neutral lipid, glycolipid and phospholipid in spot during ration experiment. Dry weight basis (± SD). Constituent percentages are of total lipids ($n = 4$).

Treatment[a]	Start				End uptake				End clearance			
	% total	% neutral	% glyco	% phospho	% total	% neutral	% glyco	% phospho	% total	% neutral	% glyco	% phospho
Control (4C)	4.2(1.1)	3.7(1.9)	51.5(2.6)	45.0(1.4)	4.0(0.3)	11.2(4.0)	46.0(0.7)	42.8(3.4)	5.8(0.4)	2.5(2.6)	50.0(2.2)	47.5(3.9)
Control (8C)	–	–	–	–	4.0(0.6)	6.1(2.7)	52.0(7.9)	41.9(5.2)	6.0(0.5)	5.0(2.2)	48.9(4.5)	46.1(2.3)
Food only (4F)	–	–	–	–	4.0(0.1)	3.7(1.8)	45.9(2.6)	50.4(4.4)	5.8(0.6)	3.7(2.5)	50.8(2.8)	45.4(3.8)
Food only (8F)	–	–	–	–	4.1(0.5)	2.6(0.3)	47.8(2.5)	49.6(2.9)	6.0(0.9)	2.7(0.7)	50.4(1.3)	46.9(1.7)
Water only (4W)	–	–	–	–	4.7(0.7)	4.1(2.3)	44.9(5.8)	51.0(3.4)	5.3(0.9)	2.6(2.9)	51.1(3.5)	42.3(6.0)
Water only (8W)	–	–	–	–	5.9(0.4)	3.8(1.0)	53.1(3.2)	43.1(4.2)	5.8(0.5)	2.6(0.1)	55.5(2.2)	41.8(2.2)
Food & Water (4FW)	–	–	–	–	3.6(0.1)	4.4(0.4)	42.4(7.6)	53.2(8.0)	6.0(0.4)	5.5(3.7)	48.9(1.7)	45.6(2.6)
Food & Water (8FW)	–	–	–	–	4.1(0.6)	4.4(0.9)	48.4(0.5)	47.3(0.5)	5.9(0.7)	5.2(2.8)	50.6(2.4)	44.2(5.2)
Food only (20F)	5.3(0.6)	4.1(0.7)	51.5(1.7)	44.4(1.9)	6.9(0.3)	4.8(1.3)	50.3(2.5)	44.9(1.2)	–	–	–	–

[a]Exposure regime. Number represents ration.

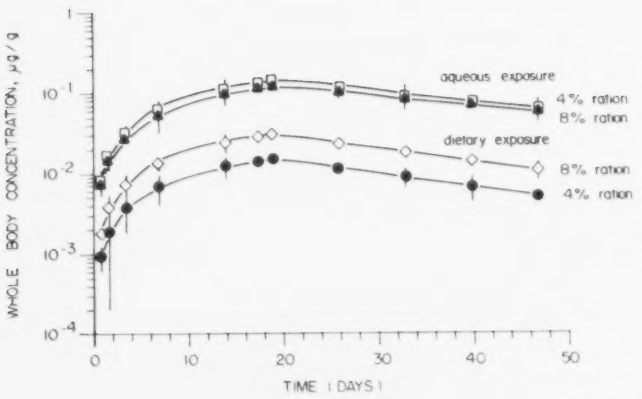

Fig. 1. Accumulation and clearance phase curves (±SD) for spot exposed to either 0.04 µg/l [^{14}C]Kepone in water or 0.101 µg/g [^{14}C]Kepone in food at two rations (19-day uptake and 28-day clearance).

of the uptake phase, while fish fed the smaller ration accumulated only 0.014 µg/g (±0.004 SE, $n=9$). In contrast, spot fed either a 4 or 8% ration of uncontaminated food and exposed to aqueous Kepone did not accumulate significantly different final Kepone body burdens (Fig. 1). The mean whole-body Kepone concentration bioconcentrated by fish fed the 8% ration was 0.117 µg/g (±0.026 SE, $n=9$); spot fed the smaller 4% ration, accumulated 0.118 µg/g (±0.027 SE, $n=9$). However, these fish were significantly smaller (Table III) and had significantly less percentage total lipids than those fed the larger ration (Table II). The clearance rate constants

TABLE III

Pharmacokinetic parameters for bioaccumulation of Kepone by spot *(Leiostomus xanthurus)*. BIOFAC predictions (± model σ).

Treatment[a]	Uptake rate constant, day^{-1}	Clearance rate constant day^{-1}	Time to half clearance, days	BCF[b] DAF	Time to 90% equil., days	Calculated equil. conc., µg/g
4F	0.273(0.020)	0.037(0.004)	18.7(2.2)	7.4(1.0)	62.0(7.2)	0.030
4W	217.3(10.2)	0.030(0.002)	23.5(1.9)	7,364(688)	78.0(6.3)	0.295
8F	0.265(0.016)	0.037(0.004)	18.7(1.8)	7.2(0.8)	62.3(6.1)	0.058
8W	185.5(9.3)	0.027(0.002)	25.5(2.3)	6,823(708)	84.7(7.7)	0.273
4FW – Food	0.262(0.023)	0.032(0.005)	21.5(3.4)	8.0(1.0)	71.0(11.0)	0.032
4FW – Water	214(25.0)	0.023(0.002)	29.9(7.9)	9,241(2681)	99.0(76.0)	0.370
8FW – Food	0.292(0.037)	0.043(0.007)	16.2(2.7)	7.0(1.0)	54.0(9.0)	0.056
8FW – Water	154(20.0)	0.020(0.006)	35.4(11)	7,868(2702)	118.0(37.0)	0.315
20F	0.235(0.061)	—	—	—	—	—

[a] Number = ration; letters = exposure; words = route in combined exposure (4FW – Food = dietary accumulation in combined exposure at a 4% ration).

[b] BCF = bioconcentration factor; DAF = dietary accumulation factor.

generated by BIOFAC are similar for the two rations, indicating little difference attributable to growth dilution (Table III). These data are in agreement with Bender and Huggett (1984) who found no relationship between fish size and lipid content and Kepone concentration in fish samples from the field. The lack of effect of ration on Kepone bioconcentration indicates that the ration effect on dietary accumulation was due to increased Kepone dosage at the larger ration and not to some physiological change in Kepone uptake due to an increase in ration alone. The similarity in uptake rate constants between fish fed the two rations also indicates little physiological difference in spot Kepone accumulation between rations (Table III).

Kepone contributions from the food and water sources were additive. The BIOFAC generated curves were similar for dietary accumulation, regardless of whether an aqueous source of Kepone was present (Fig. 2). The same is true for bioconcentrated residues in the presence or absence of contaminated food. There were no significant differences in any of these paired treatments at any sampling period (i.e., bioconcentration from combined exposure (FW) vs the single source exposure (W) in Fig. 2).

Since bioconcentrated residues were shown to be independent of ration size, the increase in contaminated food ration led to an increase in dietary contribution to final residual body burdens in spot. Dietary Kepone contributions (i.e., the concentration derived from this source divided by the total from both sources), represented 9.7% of the total Kepone body burden at the 4% ration and 18.5% at the 8% ration after 19 days of exposure. The BIOFAC model gave predictions for bioconcentra-

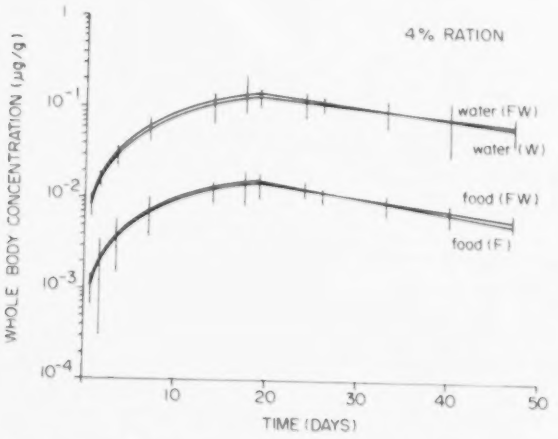

Fig. 2. Accumulation and clearance phase curves (± SD) for spot exposed to 0.04 µg/l Kepone in water and/or 0.101 µg/g Kepone in food alone or in combined exposures at a 4% ration (19-day uptake and 28-day clearance).

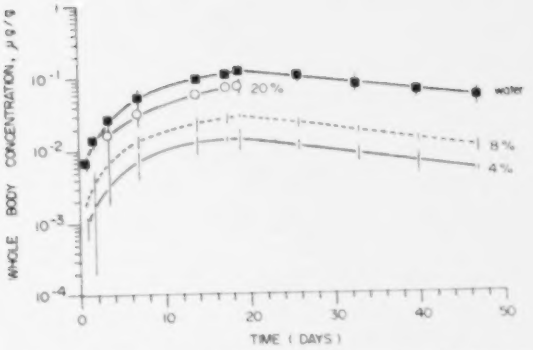

Fig. 3. Accumulation and clearance phase curves (± SD) for spot fed either a 4, 8 or 20% ration of 0.101 µg/g [^{14}C]Kepone contaminated food or exposed to 0.04 µg/l [^{14}C]Kepone contaminated water and fed uncontaminated food (19-day uptake and 28-day clearance).

tion and dietary accumulation factors which, when applied to exposure concentrations, allowed for estimates of Kepone body burdens in spot at steady state (Table III). At steady state, the dietary contribution was calculated from these values to be 9.1% and 17.5% at the 4 and 8% ration, respectively. These findings show the importance of ration size in dietary accumulation studies. When an inadequate ration is presented to test organisms, the significance of dietary accumulation of a xenobiotic may be underestimated.

Whole body concentrations of Kepone in spot fed a 20% ration of contaminated food increased significantly compared to fish fed smaller rations of the same food (Fig. 3). At the end of the uptake phase, the fish fed the 20% ration had an average of 0.072 µg Kepone/g whole-body weight (± 0.014 SE, $n = 6$). Fish fed the larger ration were somewhat larger at the end of the uptake phase than those fed the smaller rations and had a greater lipid content (Table II). As observed earlier, size and lipid content do not appear to be correlated to Kepone concentration (Bender and Huggett, 1984). Uptake rate constants for the three rations were similar, indicating little difference in uptake kinetics (Table III).

The increased Kepone body burder for fish fed a 20% ration should result in a large contribution from the dietary source if fish were exposed to Kepone in food and water simultaneously. Since it has been shown that the contributions of Kepone from the two sources are additive, and that uptake of Kepone from water is independent of ration size, it can be calculated that dietary accumulation of Kepone could account for approximately 38% of the total Kepone accumulated at this ration. This percentage represents a doubling in contributions from the dietary source with an approximate doubling of ration from 8 to 20%.

Spot fed uncontaminated food but exposed to leached or excreted Kepone in water for 19 days accumulated 0.003 µg Kepone/g from secondary uptake. This uptake represents approximately 3.3% of the Kepone accumulated by fish fed directly

on contaminated food at a 20% ration. These data compare favorably with the slight secondary uptake (1.7%) found for striped bass *(Morone saxatilis)* and PCBs by Pizza and O'Conner (1983) and indicates the insignificance of this route in determining final Kepone body burdens, even at the relatively low flow rates (15 l/h) used in the present study.

The pharmacokinetic uptake parameters calculated by BIOFAC for dietary accumulation of Kepone by spot were not affected by ration size (Table III). Thus, the 1st order pharmacokinetic principle of superposition seems to hold for dietary accumulation (Spacie and Hamelink, 1985). The average uptake and clearance rate constants for dietary accumulation were 0.269 day^{-1} and 0.037 day^{-1}, respectively, yielding a DAF of 7.26. The average uptake and clearance rate constants for bioconcentration were 201 day^{-1} and 0.028 day^{-1}, respectively, yielding a BCF of 7,094.

The clearance rate constant for bioconcentrated residues is slightly lower than that for residues accumulated through the diet. This difference may be simply a reflection of the way the BIOFAC model handles differences in analytical variability between residue samples from the two sources (i.e., final body burdens were lower for residues accumulated from the diet; therefore, there was a greater analytical variability) (Fig. 1). A relationship between route of exposure and clearance rate in fish and invertebrates has been reported for some metals, especially lead (Vighi, 1981) and zinc (Giesy et al., 1980). Clearance rate constants presented by Bruggeman et al. (1984) indicate slower clearance for residues accumulated from the water than from the food for lipophilic compounds, including decachlorobiphenyl, hexachlorobiphenyl and tetrachlorobiphenyl.

Differences in clearance rates could be related to differences in routes of exposure, since residues accumulated from the diet would come into contact more quickly with those areas thought to be important in xenobiotic metabolism (i.e., intestines and liver) (Mackie et al., 1974; Shaw and Reidy, 1979; Teal, 1977). However, Van Veld et al. (1984) showed only minimal presence of Kepone metabolites in the bile of channel catfish after two weeks of exposure to dietary Kepone. Further study concerning the metabolism of Kepone by spot would be necessary to determine the possible relationship between exposure route and clearance rate. It is unclear whether differences in clearance rate seen in the present study are significant, but the question is not trivial, since the use of first-order pharmacokinetic equations to model xenobiotic accumulation is dependent on the assumption of a clearance rate constant which is independent of exposure route.

The data from these ration size experiments indicate that dietary accumulation of Kepone by spot can represent approximately 38% of the total Kepone body burden in fish exposed to quantitatively related aqueous and dietary sources. These findings are in contrast to the conclusions of Macek et al. (1979) that dietary accumulation of Kepone in a plankton-mysid-spot food chain would be insignificant. The authors based their conclusion on Kepone work published by Bahner et al. (1977) and their

own laboratory findings on three other chemicals. There are two major problems with their conclusion. First, the chemicals that Macek et al. (1979) studied in their laboratory were not accumulated to any extent, even from water (i.e., largest BCF = 183). Second, Bahner et al. (1977) did not expose the various food chain components to quantitatively related Kepone concentrations. In fact, mysids, which served as the food source for the spot, were not exposed to aqueous Kepone at all. Since mysids can bioconcentrate Kepone from 6,000 to 13,000 times (Bahner et al., 1977), a major route of entry to spot was totally ignored. If one recalculates the contributions in the Bahner et al. (1977) study, assuming a mysid BCF of 9,500 and a more realistic spot BCF of 6,000, the dietary contributions to spot can be shown to be almost 60%.

There is no reason to question the Macek et al. (1979) conclusion for the vast majority of aquatic contaminants that are water soluble and/or metabolizable. However, one cannot dismiss the environmental importance of dietary accumulation of xenobiotics based on data from more water soluble compounds. Highly lipophilic and slowly metabolized industrial and agricultural compounds such as DDT, PCB and Kepone have caused serious environmental problems during the last few decades. This lipophilic character dictates that clearance from organisms will be slow and potential for accumulation high. Slow clearance also means that these types of chemicals will be persistent environmental contaminants. Thus, although these types of compounds represent only a small percentage of the total possible aquatic contaminants, they are of major concern in terms of the overall health of aquatic biota. According to Bruggeman et al. (1984), these more lipophilic contaminants are also the types of compounds which will have a significant food chain component and cause long term threats to higher organisms, including man.

The 24-h Kepone assimilation efficiency for spot was approximately 15%, regardless of food ration size (Table IV). These values appear low, considering the lipophilic nature of Kepone. Lipophilic organic compounds in food are expected to

TABLE IV

Mean spot [^{14}C]Kepone assimilation efficiencies as percentages of the total Kepone offered in a single feeding ± σ (n).

Experiment	To determine	Ration	Type food	Organism	Food	Filtered H_2O
3 h Assimilation	% Kepone ingested	4	Fine	17.6 ± 10.7(3)	–	–
		8	Fine	12.8 ± 0.6(2)	–	–
24 h Assimilation	% Kepone retained after 24 h	4	Fine	15.5 ± 8.5(6)	–	–
		8	Fine	14.4 ± 2.5(4)	–	–
		8	Coarse	25.3 ± 6.4(4)	–	–
24 h Assimilation	% Kepone retained no fecal reingest.	8	Fine	13.9 ± 2.3(2)	–	–
3 h Static mass balance	Partitioning Kepone to water	8	Fine	–	24.2 ± 1.1(3)	39.0 ± 1.2(3)
		8	Coarse	–	42.6 ± 3.3(3)	29.5 ± 2.2(3)

be transferred efficiently because of the relatively long term of contact between food and membranes (Mitchell et al., 1977; Pizza and O'Conner, 1983; Spacie and Hamelink, 1985). The grinding of the food in the present study to insure a more even distribution may have decreased the availability of Kepone to the fish. Static tests showed that almost twice as much Kepone remained in the food when it was more coarsely ground (Table IV). A rapid partitioning or leaching of Kepone from finely ground food to water was seen. Of the total amount of Kepone offered, only 24% remained in the food after 3 h. In contrast, partitioning of Kepone from a more coarsely ground food was less, 43% of the Kepone remaining in the food after 3 h. Thus, the assimilation efficiency was low, based on the total amount of Kepone offered, due to a poor ingestion efficiency. Comparison of 24-h assimilation efficiencies with 3-h ingestion efficiencies indicates that almost all of the Kepone ingested was assimilated, a finding more in line with the conclusions of Spacie and Hamelink (1985). These findings indicate that the dietary contribution found in the present study is most certainly an underestimate of the actual value.

Kepone residues in spot in the James River in 1982 average 0.24 μg/g whole-body, wet-weight (0.15 μg/g near the mouth and 0.37 μg/g near the turbidity maximum zone) (Bender and Huggett, 1984). Using a BCF and DAF of 7,000 and 7.4 (Table III), respectively, for spot and a BCF of 13,000 for shrimp, as determined in an as yet unpublished companion study, equilibrium body burdens for spot in the James River can be predicted. Aqueous Kepone concentrations in the James average 0.007 μg/l (Slone and Bender, 1980; Strobel et al., 1981). If an average food consumption rate of 10% is used, bioconcentration and dietary accumulation of Kepone by spot would yield body burdens of 0.049 μg/g (0.007 μg/l · 7,000 BCF) and 0.067 μg/g (0.007 μg/l · 13,000 shrimp BCF = 0.091 μg/g · 10% · 7.4 spot DAF), respectively.

These values represent fish fed only on shrimp exposed to Kepone in water. In the companion study mentioned above, we found a DAF of approximately 22 for grass shrimp. Thus, more Kepone would be available to spot which were fed shrimp exposed to aqueous Kepone and contaminated food. For spot fed shrimp that were allowed to accumulate Kepone from both sources, the concentration at steady state can be estimated to be 0.215 μg/g (0.007 μg/l · 13,000 shrimp BCF = 0.091 μg/g · 10% · 22.4 shrimp DAF = 0.204 μg/g in shrimp from food + 0.091 μg/g from water = 0.295 μg/g in shrimp from all sources · 10% · 7.4 spot DAF = 0.215 μg/g). Since dietary and aqueous sources of Kepone have been shown to be additive, this yields a predicted steady state concentration in spot of 0.264 μg/g (0.049 + 0.215), dietary contributions representing approximately 81% of the total. Even with the relatively low DAF for spot in the present study, a good estimate of Kepone tissue concentrations in James River spot is possible only when dietary contributions are taken into account.

This study indicated that aqueous Kepone bioconcentration from water was independent of food consumption rate. Ribeyre et al. (1980) have shown that dietary accumulation of xenobiotics becomes more important than bioconcentration at high

temperatures due simply to increased food consumption. Thus, any differences between Kepone concentrations in feral spot and predictions from laboratory data could be the result of differences in food consumption rates or food Kepone concentration, or both. As spot migrate into the estuary in the spring, water temperature and, presumably, food consumption rates increase, leading to greater inputs of Kepone to organisms. For a compound that is cleared slowly, this extra dietary input could lead to high body burdens that appear unrelated to aqueous Kepone concentrations. A major reason dietary contributions of lipophilic compounds have been underestimated and ignored in the past is the failure of investigators to account for feeding rates and the manner in which changes in these rates affect dietary accumulation of slowly cleared compounds.

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